816838

APPLICATION FOR UNITED STATES PATENT

Inventor(s): FUSSI, et al.

Invention: OLIGO-HETEROPOLYSACCHARIDES HAVING A HEPARIN-LIKE ACTIVITY METHOD FOR THEIR PREPARATION AND PHARMACEUTICAL COMPOSITIONS BASED THEREON.

Cushman, Darby & Cushman
1615 L Street, N.W.
Washington, D.C. 20036
Attorneys
Telephone: (202) 861-3000

SPECIFICATION

This invention relates to a hetero-polysaccharide which is susceptible of finding a therapeutical application, in general, in the prevention of thrombotic phenomena.

Another object of the present invention is to provide a method for the preparation of such a hetero-polysaccharide.

Yet another object of the present invention is to indicate therapeutical uses and pharmaceutical compositions which contain as the active ingredient the oligo-hetero-polysaccharide of the present invention.

Thrombosis is one of the most frequent factors of casualties and ailments, these latter often showing a permanent invalidity pattern in the field of the cardio-vascular ailments.

The general term "thrombosis" may include conditions displaying an exalted tendency to blood-clotting, the origins of which can be attributed to :

- "hazardous factors" originating a thrombogenic state, such as tobacco smoke, the stresses, the prolonged use of contraceptives of the progestogen type and others,
- hereditary factors, such as the lack of blood-clotting-inhibiting factors, more particularly antithrombin III,
- causative factors of various origin, sometimes not yet elucidated, such as modification of the platelet adhesiveness and others,
- factors deriving from a temporary slowing down of the blood circulation such as is experienced subsequently to surgical operations under narcosis.

The pathological after-effects consequential to a "thrombogenic" condition as caused by one or more of the factors enumerated above can be :

pulmonary, cerebral, coronaric and other thrombo-embolism,

thrombosis of the deep-lying veins,

thrombophlebites, varicose syndromes,

diffuse scattering of intravascular microthrombi.

Before so imposing a number of phenomena, it is possible, at present, to have recourse to two approaches :

1. The use of thrombolytic agents,

2. The preventative therapy of thrombogenic conditions and their after-effects. On account of the seriousness and the rapidity of the possible evolution of thromboses, it is apparent that, of the two approaches, the second one is to be preferred by far.

In order to face the thrombosis problem from the preventative angle, two classes of medicaments are now available, viz. the oral anticoagulants such as coumarin and its derivatives and heparin.

Oral anticoagulants such as coumarin and its derivatives act at the liver level and block the two blood-clotting factors proconvertin and prothrombin, but give rise to cumulative phenomena and thus lends themselves poorly to a prolonged treatment and, moreover, even though they are anticoagulants, have but a poor antithrombotic activity since they have no action on other blood-clotting factors which are closely involved in the thrombogenesis, the Xa factor and the platelet factors above all.

Heparin, under this respect, yet offers advantages in that it acts upon the several plasmatic factors of blood clotting and especially upon thrombin, the factor Xa and also on the XII factor, the XI factor and the IX factor in addition to the platelet factor called PF4. All of these actions are to be attributed to the specific ability by thrombin to unblock the inhibitor of the blood-clotting factors enumerated above, said inhibitor being present in the plasma. This inhibitor is the antithrombin III and requires, just as a co-factor to unfold its action, the

presence of heparin.

Regrettably enough, heparin has two defects : in the first place, it is active only parenterally and its effect lasts for 8-12 hours as a maximum, so that it is difficult to bring about a prolonged prophylaxis, for which 2 heparin shots daily are required. In the second place, heparin has not only an antithrombotic effect but also an anti-blood clotting action as a whole. Now, if this second effect is an asset in certain instances, in other cases the haemorrhage hazard, if the therapy is not adapted to the individual patient, becomes a serious trouble even if the prophylaxix of thrombosis offers advantages beyond any doubt.

Low mol.wt. heparin fractions are found in two cases:

(a) when depolymerizing heparin with chemical or enzymic methods (cfr. A. Horner, in "HEPARIN", Kakkar, Thomas, 1976 and Perhin and cow., Carb. Res., 18, 185 (1971)).

(b) in the mother liquors of the processes for extracting heparin for therapeutical use.

Such fractions, having a mol wt of 5,000 and containing variable amounts of sulfuric groups, generally less numerous than in heparin, have not found any useful therapeutic application heretofore.

It has now been found that such fractions, should they contain the sulfuric groups in the quantities and the positions which are characteristic of the heparin molecule, have pharmacological properties which are akin to those of heparin and therapeutical properties even improved over those of heparin.

More particularly, it has been ascertained that :

(i) oligopolysaccharide fractions coming from the depolymerization of heparin, or corresponding to depolymerized heparins having a mol wt comprised between 2,000 and 5,000 have biopharmacological properties which are improved over those of heparin, providing that they are appropriately treated so as to rebuild the active groups;

(ii) differently from heparin as such, the thus treated

fractions are active also by the oral route ;

(iii) the fractions thus treated are more readily absorbed by the skin than is heparin;

(iv) more particularly, depolymerized and reconstituted heparins are endowed with a ratio of the antithrombotic activity to the anti-blood-clotting activity which is favourable over that of the commercial heparin.

The method according to the present invention can be summarized as follows : the starting material is selected from among the heparin oligomers having a mol wt comprised between 2,000 and 5,000 and the low mol wt fractions and is treated with an equal amount by wt of sulfotrioxides of nitrogenous organic bases such as ~~piridine~~ pyridine sulfotrioxide, trimethylamine sulfotrioxide and other in an alkaline environment.

On completion of the reaction, the product is precipitated with water-miscible solvents such as ethanol, acetone and others and is taken up in an aqueous solution and purified by flowing through ion-exchange resins or molecular sieves.

From the solution the product is obtained by precipitation with water-miscible solvents or by freeze-drying.

The product thus obtained has the following properties :

Identification :

an ivory-colored powder which is slightly hydroscopic,

aqueous solution which is clear or slightly opalescent,

pH of the 5% aqueous solution : 7 to 8,

identification metachromatic reaction : 1 ml of a 2% solution of the product, added to 1 ml of a 0.0025% toluidine blue solution acidified with 0.1 ml of 1-N hydrochloric acid discharges the color from blue to reddish-blue,

specific rotatory power of the aqueous solution $[\alpha]_D^{20}$ = + 40°/+50°,

electrophoresis on cellulose acetate (~~piridine~~ pyridine/acetic

acid/water-1/10/229, pH 4.5 and development with toluidine blue) = a single band having an anodic mobility $U = 2.1 \cdot 10^{-4}$ cm2 v^{-1} sec^{-1}.

Other chemical specifications of the invention are:

Average mol wt (determined with the Somogy method in comparison with commercial heparin) : between 2,600 and 5,500 daltons.

Hexosamines after hydrolysis (reaction with carbazol): 31 ± 4%,

Organic SO_4^{--} after hydrolysis (titration with naphtharsone) : 30 ± 4%,

Molar ratio uronic acids/hexosamines/SO_4^{--} = 1/1/2.

The following Examples show particularly the method of preparation of the products according to the invention without any limitation.

EXAMPLE 1

500 g of an oligopolysaccharide having the following fundamental analytical characteristics :

pH of the 5% solution : 5.8

Organic SO_4^{--} : 13.6%

rotatory power $[\alpha]_D^{20} = +48°$

mol wt (determined with the Somogy method in comparison with commercial heparin = 4,850 ± 300 daltons,

Hexosamines : 33.5%

Uronic acids : 31.8%

Anticoagulant activity : virtually nil,

have been admixed in powder with 500 g of pyridine sulfotrioxide and 500 g of anh. sodium carbonate.

The mixture has been slurried in 10 litres of distilled water and kept stirred for 2 hrs. at room temperature.

Once that time had elapsed, the liquid has been treated with 20 litres of methanol. A white precipitate has been formed, which, separated by centrifuging, has been redissolved in 5 litres of distilled water and passed through a column (diameter 16 cm. height 110 cm) containing 20 litres of

Dowex Retardion 11 A 8.

The eluate has been adjusted to a pH of 6 with 20% sodium hydroxide and treated with 2 volumes of methanol. Upon decantation, the white precipitate has been dehydrated with methanol and dried in a vacuum at 40°C. Yield : 365 g.

The product has displayed the following properties upon analysis :

- pH of the 5% solution : 6.5
- Organic SO_4^{--} : 31%
- rotatory power : $[\alpha]_D^{20}$: +47°
- mol wt (determined with the Somogy method in comparison with commercial heparin : 5,300 ± 350 daltons
- hexosamines : 28.5%
- uronic acids : 30%
- anticoagulant activity : 36 U/mg (USP)

EXAMPLE 2

250 g of trimethylamine sulfotrioxide and 250 g of anh. sodium carbonate have been admixed, in powder form, with 250 g of an oligopolysaccharide having the following fundamental analytical properties :

- pH of the 5% aqueous solution : 6.4
- mol wt (determined with the Somogy method in comparison with commercial heparin : 3,400 ± 400 daltons
- Organic SO_4^{--} : 11.8%
- Hexosamines : 34.2%
- Uronic acids : 36%
- Anticoagulant activity (USP) : 0.5 U/mg

The mixture has been dispersed in 5 litres of dist. water and stirred 12 hours at 55°C. After this time has elapsed, the solution has been passed through a bed of 10 litres of Dowex Retardion 11 A 8. The eluate has been adjusted to a pH of 6 with 20% sodium hydroxide and treated with three volumes of acetone. A white precipitate has been formed which, after decantation, has been dehydrated with acetone and dried in a vacuum at 40°C. Yield : 165 g.

The product has shown the following analytical properties :

pH of the 5% aqueous solution : 7.1%

Rotatory power : $[\alpha]_D^{20} = +42°$

mol wt (determined with the Somogy method in comparison with commercial heparin : 3,900 ± 280 daltons

Organic SO_4^{--} : 28.5%

Hexosamines : : 29%

Uronic acids : 30%

Anticoagulant activity : 17 U/mg (USP)

The product obtained with the method described above has been subjected to assays to ascertain its pharmacobiological properties and its activity.

Toxicological tests :

No toxic effects when administered orally to rats, mice, rabbits and Guinea pigs up to a dose of 1,000 mg/kg b.w.

LD_{50} i.p. (mice) more than 3,000 mg/kg b.w. : LD_{50} i.v. (mice) : more than 1,000 mg/kg b.w.

LD_{50} i.p. (rats) about 2,000 mg/kg b.w. LD_{50} i.v. (rats) 354 mg/kg b.w.

Clarifying activity test :

The product lowers the seral levels of the triglycerides considerably in animals affected by experimental hyperlipaemia from Triton.

Anticoagulant activity :

USP equal to, or more than 50 U/mg

Kaolin-Cephalin clotting time test (KCCT) : 7 - 19

Ratio of antithrombotic activity to anticoagulant activity in vitro (Yin's/KCCT) : 2.5.

In vivo (dogs) antithrombotic and anticoagulant activity :

The product, administered intravenously (i.v.) (25 IU/kg) and orally (300 - 1500 U/kg) extends the thrombine time and the KCCT, and protects against thrombosis as induced by thromboplastines.

In vivo (rabbits) antithrombotic activity .

The product administered intravenously at the dose of 20 Anti Xa U/kg protects from thrombine-induced thrombosis.

Thus, the following predictable therapeutical uses are suggested, either orally or parenterally :

prevention of post-operatory thromboembolisms

prevention of thrombotic seizures consequent to a thrombogenic conditions such as for example that which occurs in fertile women when treated for a long time with oral contraceptives of progestogenic type

prevention of venous thromboses

prevention of hypercoagulability states

correction of the hyperdislipaemic states (hyperdislipoproteinaemias).

We claim: